

group of ten Zone A adults without chloracne (however, a sample of this group was lost), and ten subjects from the surrounding noncontaminated area. It should be pointed out that these are the first subjects whose results of laboratory medical examinations and individual clinical histories (from 1976 through 1985) have been associated with quantitative measurements of TCDD levels in samples of blood taken near the time of acute exposure.⁷⁹

The lipid-adjusted levels of serum TCDD for the three groups were determined. Detected TCDD ranges in children with chloracne and in adults without chloracne were 830–56,000 and 1800–10,000 ppt, respectively. Although any comparison may be biased by age-related factors, based on groups, chloracne appears to be associated primarily with higher TCDD levels. However, it is also remarkable that blood lipid TCDD levels as high as 10,000 ppt following acute exposure are compatible with the absence of such a skin disorder. Among the ten nonexposed subjects, only one had detectable levels of TCDD (~140 ppt), which possibly resulted from a <1% carryover from a preceding assay of a sample with a high level of TCDD.

7. EARLY HEALTH FINDINGS

Medical examination programs were initiated with the following aims: to ascertain early adverse health consequences in the exposed population, to give guidance for the allocation of service and resources, to identify needs and suggest areas for further surveillance and research.

The earliest sign of adverse effects in humans became apparent when, on the sixth day, 19 children were admitted to local hospitals with skin lesions caused by contact of uncovered parts of the body with caustic chemicals contained in the cloud.

7.1. Chloracne

By the end of 1976, 34 cases of chloracne were diagnosed in persons under 15 years of age. It was then decided to examine all children attending nurseries, infant and primary school of the contaminated areas. Nearly 90% of them had skin examinations. By April, 1977, 187 cases of overt chloracne were diagnosed by an expert panel, and 164 (88%) were in children. Chloracne distribution closely resembled the TCDD contamination pattern (Table 8). In Zone A, 61 cases were observed, of whom 19 were adults. The 42 cases among children corresponded to 19.6% of all children living in this Zone, indeed a large proportion, which was even higher when only the most contaminated subzones were considered. The prevalence of chloracne was 0.5% in Zone B, and 0.7% in Zone R. This distribution of chloracne cases provided also the first hint of a possibly inaccurate definition of the boundaries of contamination zones. As a matter of fact, in certain parts of Zone R in the municipalities of Seveso and Meda, the proportion of chloracne in children was higher than in Zone B. Thus, for example, in the Zone R suburb of Polo, located in the top right-hand corner of the accident scenario, southeast of the plant (Fig. 1), out of 750 children, 19 (2.5%) were diagnosed as having chloracne. Fifty-one cases occurred in children residing outside

Table 8

Distribution of 164 Chloracne Cases Diagnosed up to April, 1977, in Children Having Their Residence in Different Areas of the Contaminated Territory or Outside

Area	Total population aged 3-14 years	Chloracne cases	Percent
Zone A total	214	42	19.6
Zone A max. ^a	54	26	48.1
Zone B	1,468	8	0.5
Zone R	8,680	63	0.7
Zone R Polo ^b	750	19	2.5
Other	48,263	51	0.1

^aIncludes only the most contaminated part of Zone A

^bSubzone located near the plant

the designated contamination zones. Where they were at the time of the accident could not be established with certainty. By mid-1978, six additional cases were detected, bringing the total number to 193. No further cases of chloracne were discovered or reported after this time.⁸⁰⁻⁸²

The higher frequency of chloracne in children than in adults is possibly explained by the fact that the former had more opportunities to come in contact with the toxic cloud components and to ingest or inhale them through outdoor activities, contact with soil, vegetation, dirt outside their homes, and so forth. Other relevant explanatory factors might be the absence of a systematic screening in adults, which possibly left unnoticed other existing cases, or a difference in susceptibility to dioxin effects at different ages.

7.2. Subjective Symptoms and Laboratory Tests

The comparison of 146 cases and 182 controls (nonchloracne children, age and sex matched to the cases, selected from the area) revealed a significantly higher frequency of nausea, lack of appetite, vomiting, abdominal pain, headache, and eye irritation among the former. In addition, they exhibited a higher frequency of abnormal values for the liver enzymes gamma-glutamyltranspeptidase (GGT) and alanine aminotransferase (AAT), and for urinary aminolevulinic acid (ALA-U), a porphyrin precursor. Chloracne subjects from Zone A had biochemical abnormalities more frequently than those from other zones.⁸⁰

Health examination results of 18 subjects over 14 years of age affected by chloracne were also reported (no control group was concurrently examined). There was a high frequency (around 25%) of self-reported symptoms, and of signs of liver enlarge-

ment. In 1977, biochemical tests showed elevated serum cholesterol values of higher than 230 mg/100 ml in eight subjects, and ALA urinary concentration outside the reference range in five subjects.⁸³

7.3. Peripheral Neuropathy

Peripheral neurological changes were among the signs of TCDD toxicity. Persons evacuated from Zone A were invited to undergo neurological examination in 1977 and 1978. A nonexposed population served as reference. Electrophysiological and clinical signs of peripheral neuropathy were nearly three times as frequent among Seveso residents having either raised serum liver enzyme levels or chloracne (12/55 or 22%) than among controls (13/168 or 8%). When only subjects younger than 20 years with chloracne were considered, the relative frequency rose up to nearly five times.⁸⁴

7.4. Enzyme Induction

In the early months after the accident, urinary D-glucaric acid excretion, an indirect index of enzyme induction, was measured in 14 children with and 17 children without chloracne, all from Zone A. The former had a significantly higher level of D-glucaric acid in urine.⁸⁵

It is understandable how in the hectic, early postaccident period these observations were often lacking formal design and proper conduct. For example, it was not easy to control biases, such as those linked to interview and reporting, or assure standardization of diagnostic procedures, proper selection of controls, adequate size of the sample, and so forth. Notwithstanding these limitations, the reported early surveys, which mainly concerned chloracne cases, showed beyond any doubt that at least a portion of the population had been exposed to the powerful toxin 2,3,7,8-TCDD.

8. SURVEILLANCE PROGRAMS

Surveillance programs were then designed in order to continue over time the health monitoring of those subjects exhibiting immediate, acute effects (e.g., chloracne cases), and identify in the affected population at large the possible occurrence of health effects in the short- to mid-term period. One of the major problems was the large size of the population in the active surveillance. This difficulty was augmented by the lack of a preexisting validated information system and by the time constraints which led many different teams to be called into the area, with diminished possibilities for quality control and procedure standardization.

8.1. Spontaneous Abortion

According to animal data, an increase of spontaneous abortions in pregnant exposed women was to be expected. Ascertainment of spontaneous abortion is, in general, a difficult surveillance task. Specific problems further complicated the picture in our case. One was the absence of a valid, on-going data collection system; another involved the moral and political issues related to legalization of abortion; and, finally, a very active birth control campaign was carried out which may have decreased conception rates. In such a context, the completeness, accuracy, and quality of data remained questionable. Nevertheless, several attempts were made to report and interpret the occurrence of spontaneous abortions in the area.

Analysis by trimester (from the accident to early 1978) and by municipality showed some time-related variability with the highest abortion rate seemingly occurring in the earliest trimester in the contaminated areas. However, it was difficult to determine the possible contribution of TCDD exposure, or of exposure to a "chemical disaster" as such, and to exclude a major role of biases related to information recording and data collection, etc. Results were considered inconclusive, if at all valid.¹⁸

In another report,⁸² crude estimates drawn from vital statistics sources were provided. A decrease in the birth rate was observed in 1977-1980 in the entire Lombardy region, and not just in the accident area. The proportion of abortions compared to live births per year was not considered to depart from "the generally accepted abortion rate of 10-20%."

In a third analysis, notifications of spontaneous abortions to county medical officers were used. Spontaneous abortion rate in 1977 was higher than in 1976, but not departing from historical rates estimated from 1973 onwards. An improved physician's care in notification was considered a probable explanation for the change in the postaccident period. These data were then supplemented with information directly provided by hospitals in the region (Table 9). An increased pregnancy loss percent was seen in late 1976, with a subsequent fall (again, a change in physicians' notification attitude?) Zone B rates showed a further increase in mid-1977, and were consistently higher than those of Zone R and the noncontaminated area; the excess was statistically significant only in the third trimester of 1977. Data quality was considered questionable, and results lacked consistency.⁸⁶

8.2. Cytogenetics

The first chromosome analysis was performed in 1976 at the request of hospitals where eight children aged 2 to 10 years and four pregnant women with skin lesions presumably caused by TCDD exposure had been admitted. For comparison, the earliest available results on ten unmatched subjects were adopted. The proportion of aberrant cells was higher in the exposed children and women, but only when gaps were included.⁸⁷

A more extensive cytogenetics study included 301 subjects, as indicated in Table

Table 9
Pregnancy Loss Rate [Abortions/(Births + Stillbirths + Abortions) \times 100] by Exposure Zone and Trimester^{a,b}

Zone	Jul-Sep 1976	Oct-Dec 1976	Jan-Mar 1977	Apr-Jun 1977	Jul-Sep 1977	Oct-Dec 1977
B	No. of abortions 3 Pregnancy loss % 11.1	4 22.2	5 17.2	8 28.5	10 31.2	4 13.7
R	No. of abortions 19 Pregnancy loss % 13.7	17 16.3	15 12.7	17 12.5	16 11.4	20 13.8
Noncontaminated	No. of abortions 74 Pregnancy loss % 11.0	94 14.8	119 16.6	81 13.0	67 10.5	99 14.3

^aSource: Special Office for Seveso, Lombardy Region

^bZone A is not shown because very few pregnancies occurred

Table 10
Cytogenetic Study in Seveso, 1977. Frequency of Chromosomal Aberrations in Lymphocytes

Exposure	No. of subjects	No. of mitoses	% aberrant cells	
			Including gaps	Excluding gaps
Acute	145	6470	2.49	0.99
Chronic	69	3040	2.53	0.92
Controls	87	3958	1.64	0.48

10. Those with acute exposure were subjects living in the most contaminated area near the accident plant; workers employed in that plant were considered as having had long-term ("chronic") exposure, age- and sex-matched controls were people living in the surrounding noncontaminated area. Proportion of aberrant cells was higher among the exposed, but statistical analysis showed that the only significant difference was among the scorers of the five laboratories involved in the survey. A further analysis of a larger number of mitoses on selected samples from the three exposure categories was then carried out. Differences between exposure categories did not become significant even after correction for interobserver variability. No consistent evidence of chromosomal effects associated with TCDD exposure was thus provided by this study.⁸⁷

A third set of data was obtained after examining maternal peripheral blood, placenta and umbilical cord, and fetal tissues of induced abortions in 19 women from the Seveso area, and 16 women not known to be exposed to environmental mutagens and teratogens who had abortions for nonmedical reasons. Within a pattern of marked variability, no significant differences were observed in the frequency of individual types of aberration, average number of aberrations per aberrant cell, or frequency of polyploids in maternal blood and placenta between the two groups. Instead, fetal tissues of exposed pregnancies exhibited aberrant cell frequency significantly higher and a greater number of aberrations per damaged cell than control pregnancies. Several factors might explain these findings, including those related to growth in culture. In addition, the possibility of preexisting chromosomal damage in fetal cells could not be ruled out. No differences were seen regarding ~~these~~ pregnancies started in Seveso before versus those after the accident. Thus, the extent to which the increased frequency of chromosomal aberrations in fetal tissue reflected maternal exposure to TCDD could not be established.⁸⁸

8.3. Birth Defects

The first set of data available consisted of 30 cases of induced abortion (of which 3 were from Zone A, and 5 from Zone B), and four spontaneous abortions (from Zone R), all of which occurred in 1976 after the accident. Embryological and histomorphological investigations were conducted on this material, and no indications of

Table 11
Relative Risk and 90% Confidence Interval
for Selected Groups of Malformations
in the TCDD-Contaminated Area (Zones A, B, and R)
versus the Surrounding Noncontaminated Territory

	1977-1982	First quarter 1977
Total defects	0.97 (0.83-1.13)	1.49 (0.64-3.45)
Major defects	0.83 (0.67-1.04)	0.93 (0.26-3.32)
Mild defects	1.14 (0.92-1.42)	2.50 (0.79-7.94)

mutagenic, teratogenic, or fetotoxic effects attributable to TCDD were detected. In 23 induced abortions, no anomalies or organic alterations could be found. In six other cases, various morphological alterations were visible; some were probably artifacts, and some were of borderline pathological significance. The four spontaneous and the one remaining induced abortions were probably related to dioxin exposure, but this link could not be proved. Investigations were limited by the fact that in the majority of cases fetal tissues were incomplete.⁸⁹

At the beginning of 1977 a congenital malformation registry was established, which included all live births and stillbirths to women residing in the accident area in July, 1976. Data were collected for the period 1977-1982; 742 malformed infants were registered out of a total of 15,291 births (live and still). Out of 26 births in Zone A, no cases of major malformations were found. In none of the three exposure zones (A, B, or R) was the frequency of mild, major, or combined defects significantly higher than in the reference population. Table 11 shows relative risks for the entire surveillance period and for the first quarter of 1977, when children had probably been exposed to TCDD during the first week of gestation (140 births in all). None of the relative risk values were statistically significant. Major information biases were excluded, whereas the small number of exposed pregnancies, especially in Zones A and B, might have precluded the identification of low-risk and/or very rare defects.⁹⁰

A piece of related information came from the mortality and cancer incidence studies (dealt with in more detail below). Mortality related to congenital malformations was not increased among those born after the accident.⁹¹ In the same period, for none of the cancer types examined was the incidence in the exposed young population significantly increased.⁹²

8.4. Follow-Up of Special Groups

Long-term effects on the *peripheral nervous system* (PNS) were explored in 152 chloracne cases who agreed to participate in a survey conducted between October, 1982, and May, 1983, and in 123 subjects without chloracne, frequency-matched by sex and age, who volunteered to serve as the reference group. None of the subjects had

a clear-cut peripheral neuropathy, but clinical and electrophysiological signs of PNS involvement were, significantly, nearly twice as frequent in the chloracne group than in controls. In particular, there were 11 cases versus 2 controls presenting at least two bilateral clinical signs, and 25 cases versus 9 controls exhibiting at least one abnormal electrophysiological function.⁹³

Forty-eight children aged 3 to 8 years from Zone A underwent repeated examinations from November, 1976, to May, 1979, for the study of *immunologic effects*. Control subjects were selected from the school population of a nearby noncontaminated town. Total serum complement hemolytic activity (CH50) had significantly higher values among the exposed subjects at each examination. Exposed children also exhibited higher values for lymphocyte responses to phytohemagglutinin (PHA) and to pokeweed mitogen (PWM), and in the absolute number of lymphocytes of peripheral blood. Results for other tests failed to show clearly diverging values between the exposed and control subjects. Consistently increased values were more evident in children with chloracne.⁹⁴

Induction of microsomal enzymes in the liver was one of the best documented TCDD effects in laboratory animals. An indirect test of enzyme induction, urinary D-glucuronic acid, was evaluated between 1976 and 1979 in different groups of the exposed population and controls. Children from Zone A with chloracne had, in 1976, significantly higher levels of D-glucuronic acid in urine than children without skin lesions. In 1979, children who had left Zone A had levels similar to those of controls, whereas in children still living in Zone B the urinary excretion was significantly higher. In 1980, however, urine samples of the Zone B children showed significantly lower levels. In 1981, 34 children evacuated in 1976 from Zone A had normal values, whereas 61 children from Zone B and 59 children from a Zone R sector very close to the plant (i.e., Polo) had urinary D-glucuronic acid levels almost significantly elevated. Adults living in Zone B ($N = 117$) had significantly higher levels of D-glucuronic acid excretion in 1978 than controls ($N = 127$) from a noncontaminated area.⁸⁵

Children from the three contaminated zones were followed from 1976 to 1982 to examine whether *liver function* and *lipid metabolism* showed alterations as possible consequences of TCDD exposure. In all, nearly 400 children aged 6 to 10 years at the time of the accident were examined on a yearly basis. The only clear-cut difference in test values between exposed and reference children was seen in 1976 and 1977, when boys living in the most contaminated Zone A exhibited consistently higher levels of GGT and AAT. The increase was slight, perhaps attributable to TCDD exposure, and disappeared with time. No alterations of blood concentrations of cholesterol and triglycerides were found.⁹⁵

Repeated surveys on the group of 193 *chloracne cases* and on unexposed control subjects matched for age and gender were conducted until 1985, with a participation rate between 70 and 80%. No significant differences or temporal trends in mean values of liver enzymes and lipids were detected. A decrease in cholesterol and triglyceride values was apparent in the chloracne group between 1976 and 1982. At the end of the follow-up in 1984, one subject had persistent chloracne, and five had chloracne scars on face and forearms. Motor and sensory nerve conduction velocity was measured, and

neither significant differences between groups nor temporal trends were observed. Apart from skin signs, no clinical or systemic sequelae of chloracne were thus detected 8 years after first exposure.⁹⁶

Another special group surveilled was comprised of *workers* employed in *decontamination operations* in the area. These people entered the most contaminated part of Zone A under strict personal protection and environmental measures. They underwent preemployment medical examination for eligibility. The values of a set of preemployment tests (e.g., liver function, lipid and heme metabolism) were compared with the same values after 9 months, and with those of an unexposed group. No significant changes were detected.⁹⁷ Later analysis of cleanup workers' experience confirmed that, on average, the safety measures taken had been effective. No TCDD-related clinical health impairment was found (as, for example, chloracne, liver impairment, peripheral neuropathy, porphyria cutanea tarda), and no significant differences in biochemical outcomes compared with unexposed subjects were detected. Nine subjects left for non-health-related reasons, and five for negative job fitness evaluation; for two of them a transient effect of exposure to TCDD could not be completely excluded.⁹⁸

Between 1976 and 1985, laboratory tests were periodically performed on the 20 subjects whose serum was assayed for TCDD, to detect possible alterations of the liver, kidney, bone marrow, lipid metabolism, and immune system function. However, only modest, transient, small departures from the normal ranges were observed in four children with chloracne, four Zone A adults, and one referent subject. None of the observed alterations had pathological significance either with respect to the number of tests involved or with respect to the extent of the alteration.⁷⁹

9. LONG-TERM MORTALITY AND CANCER INCIDENCE STUDIES

All surveillance programs were supervised, and their results periodically evaluated by an International Steering Committee which ended its work in 1984. Their conclusion was that chloracne represented the only health outcome clearly attributable to the accidental exposure to TCDD. No conclusion could be reached at that time regarding long-term effects. Surveillance programs were discontinued, but long-term investigations were designed in order to examine mortality and cancer incidence.

As time passed, it appeared that there might be a migration away from the area by those people who had most suffered physically, emotionally, or economically because of the accident; and they may well have been the most relevant to the determination of late health effects of accident exposure. In addition, a dilution phenomenon related to the moving out of exposed and moving in of nonexposed subjects was to be expected. In order to avoid these sources of bias, a cohort approach was adopted. The study population was thus comprised of all persons ever resident in one of the 11 towns within the accident scenario, at any time from the date of the accident onwards (including newborns and immigrants), irrespective of their current residence. The information about towns and street addresses allowed attributing subjects to one of the three exposure

zones or to the surrounding noncontaminated area. Admission into the study cohort was discontinued as of December 31, 1986; no potential for exposure was deemed to exist any more for newcomers into the area after that date.

The follow-up was based on individual information recorded on vital statistics registries which are maintained by every municipality in Italy. When a person moved outside the study area, the towns concerned were contacted successively, until the person was located. The tracing turned out to be successful for over 99% of study subjects, and for them vital status and, when deceased, cause-of-death information became available.

Because of the absence of a national registration system of cancer cases, the cancer incidence study had, instead, to be limited to people residing within the Lombardy region (nearly 9,000,000 inhabitants). The linkage of the information on all hospitalizations in the region with the records of cohort members allowed the identification of the study subjects admitted/discharged with a diagnosis mentioning cancer. Original medical records were reexamined to ascertain true diagnosis and date of occurrence of cancer. The ascertainment rate for cancer morbidity was close to 95%.

People living in the territory surrounding Zone R, not contaminated by TCDD, were the source of reference data. They shared with the index population the main characteristics related to living and occupational environment, personal habits, and social and educational background.

Results for mortality in the 10 $\frac{1}{2}$ -year period following the accident have been reported.⁹⁹ A statistically significant increase in mortality from chronic ischemic heart disease was noted in males in the early postaccident period. The increase was highest [relative risk (RR) = 3.2] in Zone A. Among females, an increased pattern of cardiovascular mortality was noted (RR in Zone A = 1.9). Two mechanisms have been hypothesized to explain the increased pattern, i.e., TCDD toxicity and postdisaster stress which might have precipitated preexisting conditions. Both may have contributed.¹⁰⁰ The relative risk for all cancer deaths was slightly below 1. Among males, suggestive increases were seen for soft tissue sarcomas, melanoma, and myeloid leukemia.

The mortality study was descriptive in nature, hence results did not permit conclusively associating any of the unusual cancer mortality findings with exposure to TCDD in 1976. Other limiting factors were the short time period elapsed since first exposure, the small number of deaths from certain causes, and exposure definition based on soil TCDD levels rather than on individual biological indicators.

Cancer incidence findings for the first postaccident decade are available and are summarized in Table 12. Cancer cases in Zone A were too few (seven cases among males and seven among females) to elicit any meaningful conclusion. In Zone B, where the relative risk for all cancers was exactly 1.0, four specific relative risks attracted attention: two were increases (hepatobiliary tract and hematopoietic tissue) and two decreases (breast and uterus). Liver is certainly one of the target organs of TCDD toxicity. The noted hepatobiliary increase was mainly sustained by primary liver cancer in men (four cases, RR = 2.1; confidence interval 95%, CI₉₅ = 0.8–5.7), and by extrahepatic bile ducts and gallbladder cancer in women (four cases, RR = 4.8, CI₉₅ = 1.7–13.5, statistically significant). Another suggested site of TCDD action is

Table 12
Cancer Incidence, 1977-1986, in the Population Aged 20-74 Years Living
in the TCDD-Contaminated Area: Results for Selected Cancers
among Males and Females Combined

Cancer site	Zone B			Zone R		
	Cases observed	RR ^a	CI ₉₅ ^b	Cases observed	RR	CI ₉₅
All cancers	115	1.0	0.8-1.2	790	0.9	0.9-1.0
Digestive system	30	1.0	0.7-1.4	211	0.9	0.8-1.0
Hepatobiliary tract	10	2.3	1.2-4.4	23	0.7	0.5-1.1
Respiratory system	24	1.0	0.7-1.5	163	1.0	0.8-1.1
Soft tissue sarcoma	0	—	—	8	2.3	1.0-5.1
Skin	5	0.9	0.4-2.1	41	1.0	0.7-1.4
Breast ^c	10	0.7	0.4-1.3	113	1.1	0.9-1.3
Genitourinary	18	0.9	0.5-1.4	133	0.9	0.7-1.1
Uterus	2	0.4	0.1-1.5	23	0.6	0.4-0.9
Hematopoietic tissue	15	2.1	1.2-3.5	45	0.9	0.6-1.2
Hodgkin's disease	3	2.6	0.9-9.0	7	0.9	0.4-2.0
Non-Hodgkin's lymphoma	4	1.6	0.6-4.3	23	1.3	0.8-2.0
Multiple myeloma	4	3.9	1.4-1.7	4	0.5	0.2-1.4
Myeloid leukemia	3	2.8	0.9-9.0	7	0.9	0.4-2.1

^aRR, relative risk.

^bCI₉₅, 95% confidence interval.

^cFemales only.

hematopoietic tissue. The increase in Zone B was statistically significant, and consistently affected males and females (RR = 2.3 and 1.9, respectively). In particular, lymphoreticulosarcoma among males (three cases, RR = 5.3, CI₉₅ = 1.6-17.5), and multiple myeloma among females (two cases, RR = 5.1, CI₉₅ = 1.2-21.6) showed statistically significant increases.¹⁰¹ Thus, the population of Zone B exhibited the clearest suggestions of a possibly increased cancer occurrence, a finding that might be consistent with their postaccident experience (they remained in the contaminated area, and their compliance to restrictive regulations was never evaluated). The low incidence of estrogen-dependent cancers (breast and uterus) was also of great interest since TCDD is known to exert a powerful antiestrogenic action.¹⁰²

The remarkable result in Zone R was, instead, the elevated risk of soft tissue sarcomas, along with the significantly lowered risk of uterine cancer. Soft tissue sarcoma is another tumor associated by several investigations with TCDD exposure.¹⁰³ The increase noted in Zone R was twofold, and of borderline statistical significance.

Relevant to the hypothesis of an association of the noted cancer occurrence pattern with TCDD exposure was the fact that the largest increases for hepatobiliary cancer, hematologic neoplasms, and soft tissue sarcomas, as well as the most striking decreases for uterus (mainly corpus uteri) and breast cancer, were estimated among people residing for the longest period in the contaminated area.¹⁰¹

Table 13
Cancer Incidence, 1977-1986, in the Population Aged 0-19 Years Living
in the TCDD-Contaminated Area: Results for Selected Cancers

Cancer sites	Cases observed	Cases expected	Relative risk	95% confidence intervals
All	23	18.2	1.26	0.8-2.0
Ovary and uterine adnexa	2	0.0	—	—
Nervous system	5	3.4	1.45	0.5-3.9
Brain	4	3.0	1.32	0.4-4.0
Thyroid ^a	2	0.4	4.66	0.7-33.1
Non-Hodgkin's lymphoma	2	1.3	1.54	0.3-7.6
Hodgkin's lymphoma	3	1.9	1.54	0.4-5.7
Lymphatic leukemia	3	2.8	1.07	0.3-3.7
Myeloid leukemia	2	0.9	2.30	0.6-9.2

^aCases are restricted to females

The above results concern people 20-74 years of age. Mortality and cancer incidence of the young members of the cohort (1-20 years of age) were analyzed separately. Mortality data showed an increase of leukemia deaths above expectations, although statistically nonsignificant, in both males and females. A suggestive increase of congenital anomalies was also noted; however, five out of the seven observed anomalies in the contaminated area turned out to have occurred in children born before the accident.⁹¹ Cancer incidence was only slightly above expectations. Given the small number of events, results are presented in Table 13 for the entire contaminated area (A + B + R). Of some interest were the increased risks for thyroid cancer and myeloid leukemia, even though statistically nonsignificant. The suggestion of a possible association between exposure to TCDD and thyroid cancer finds support in experimental and human data. The increased risk of myeloid leukemia is quite consistent with the findings in the adult population.⁹²

Once again, results of this analysis should be viewed with caution because of the limitations of the study, in particular the very limited number of events involved and the absence of information on individual exposure.

To interpret these interim results of the long-term investigations, one can note that in Zone A, where inhabitants certainly had a heavy, short-term (few weeks) exposure, the population size was small, and the study had no power to detect rare events. No cases of cancer that could be considered as "sentinel" events occurred. In Zone B the population was larger, and hence there was greater power of the study, while exposure was on the average lower, but potentially much longer than in Zone A. Here, two types of cancer known to probably be associated with TCDD exposure were in excess (hepatobiliary and lymphopoietic), and one can hypothesize that a balance of exposure intensity and sample size was probably achieved in this population subgroup. Another very rare tumor possibly associated with TCDD (i.e., soft tissue sarcoma) was, instead, increased in the largest but least contaminated area (Zone R). Taken together,

these results support the hypothesis of an increased, albeit modestly so, cancer risk, and certainly motivate further investigations. However, they do not prove conclusively that an association with TCDD exists. In fact, results only refer to the first postaccident decade. Second, exposure assessment was based on environmental measurements only, and exposure categories were based on official residence within zones whose boundaries seemed, at least in part, debatable. Finally, because of the rarity of the health outcomes of interest, this relatively large study suffered from sample size problems.

10. OVERALL INTERPRETATION AND PERSPECTIVES

Overall, three sets of health results are available, which are summarized in the Appendix. Their interpretation is limited by the weaknesses inherent in several studies.

Results of short- and mid-term surveillance programs clearly documented that the accident caused toxic damage to the population. Chloracne was the only health effect consistently established. Other health outcomes known to be possibly associated with dioxin exposure were investigated. For none of them could an unusual pattern, either for the frequency or for the type of outcome concerned, attributable to TCDD be firmly established. These investigations, however, suffered from all of the constraints related to a postdisaster scenario, which often precluded proper design (e.g., lack of referent subjects), efficient conduct (e.g., compliance to the scheduled examinations), and valid conclusion (e.g., information and selection bias).

The second set of data came from the follow-up of small, selected groups, mainly chloracne children. Results failed to show health impairment in these heavily exposed subjects up to 8-9 years after the accident. In addition, they showed that chloracne is reversible and not necessarily associated, at least in the time span considered, with other systemic effects. Longer follow-up and larger size are necessary to corroborate these conclusions. Moreover, results in these few subjects cannot be easily generalized until susceptibility factors are taken into consideration.

The last set of data are provided by early results of long-term investigations. After 10 years, these investigations uncovered an increase of cardiovascular mortality early after the accident possibly explicable in terms of precipitation of preexisting diseases caused by the stressful experience following the chemical disaster. In addition, a slight departure from the background occurrence of certain tumors was observed. All cancer departures from expectations are consistent with previous experimental and human data and are plausible from a biological perspective. However, the small number of events, and the still short observation period prevent sound conclusions. An additional limitation is exposure classification based on soil contamination data which do not actually indicate exposure and body burden.

The Seveso cohort provides unique opportunities for the investigation of TCDD effects in humans. In fact, it is the largest observable group of persons with documented special exposure to TCDD with the possible exception of Agent Orange in Vietnam: the blood levels measured are the highest so far reported, and exposure seemed relatively free of other known or persistent contaminants.

A great deal of work is still to be done, and research plans are being adjusted to reflect the rapidly increasing knowledge of TCDD toxicity, mode and mechanisms of action, environmental movement, targets, and so forth. Three research paths may be particularly rewarding and are presently being pursued.

The first is related to the fact that time since initial exposure was too short to reveal a clear pattern of possible TCDD cancer effects. Accordingly, the study will continue so as to examine the health experience of the population for at least two full decades after the accident.

The second path stems from the fact that exposure to TCDD probably varied among people even within the same contamination zone. Thus, zone categorization did not reflect either in absolute or relative terms the actual level and difference of TCDD exposure. A better definition of individual exposure would greatly improve interpretation of cancer findings. To this end, in a sample of the population, the association with TCDD of the cancer types which exhibited an increased incidence will be studied in more depth with a case-control approach, aimed particularly at better qualifying and quantifying exposure (also with the use of biological markers) such as blood dioxin measurements, and controlling sources of confounding or modification of the effect (e.g., occupation, diet, personal habits).

Meanwhile, TCDD levels in the blood of Seveso subjects are being measured from previous specimens and additional samples are expected to be measured.⁷⁸ Recently, Needham *et al.*¹⁰⁴ presented some preliminary findings concerning Zone B residents: since detected TCDD levels appeared to be approximately 5–20 times as high as background, the conclusion was drawn that in 1976 some of these residents were heavily exposed. In addition, subjects who immigrated into Zone B after July of 1976 were tested for TCDD in their blood after some years of residence: the levels detected were comparable with background.

A third promising study concerns the existence of a genetically determined inter-individual variability modulating TCDD effects in human cells. In particular, to evaluate the role of susceptibility factors in determining the cancer risk, cancer cases and controls with the same potential for exposure will be studied and examined with a variety of laboratory assays (including Ah receptor, gene markers of polymorphism and inducibility, blood TCDD.)

These studies are foreseen to last for a long period of time, and are expensive and complex. A sensible question is: are they worth conducting? Our answer is yes, if one considers that TCDD is now known to be a widespread contaminant of our environment and represents one of the major environmental health problems in industrial countries. An improved knowledge of its effects on humans will constitute an important contribution to the protection of human health and quality of our environment.

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APPENDIX: SYNOPSIS OF THE HEALTH INVESTIGATIONS PERFORMED ON THE POPULATION POSSIBLY EXPOSED TO TCDD FOLLOWING THE SEVESO, ITALY, ACCIDENT IN 1976

Population	Outcome	Results (references)	Period	Remarks
Dermatologic screening of some 16,000 children aged 0-14 years. Adults spontaneously reporting (for examination)	Chloracne	A total of 187 cases ascertained (164 aged ≤ 14 years). Strong positive association of frequency and severity of cases with soil TCDD level. 20% of Zone A children affected. ⁸⁰⁻⁸²	1976-77	Seven additional cases diagnosed during 1977-78. No further cases after 1978.
Children with chloracne ($n = 146$), controls ($n = 182$)	Symptoms and lab tests	Increased frequency of GI symptoms, headache, and eye irritation. Higher frequency of abnormal GGT, GPT, and ALA-U. ⁸⁰	1977	Probable interview/reporting bias for symptoms. Controls not properly selected.
Some 300 residents evacuated from Zone A and some 300 referents	Peripheral neuropathy	Two- to threefold increased frequency of abnormalities among subjects with signs of TCDD exposure (chloracne or increased liver enzyme levels). ⁸⁴	1977-78	No acute polyneuropathy found. All nonsevere cases.
Women admitted to obstetrics departments in Lombardy and residing in the polluted area	Spontaneous abortions	No clear changes in pregnancy loss rate by trimester or towns of residence. ¹⁸	1976-78	No historical data for comparison. Poor data quality.
Census and vital statistics data	Spontaneous abortions	No apparent increase. ⁸²	1977-80	Indirect estimate. Possible reporting bias.
Pregnancies in the contaminated area	Spontaneous abortions	Slight increase in 1977 compared with 1976. Abortion rates lower than external reference. Internal comparison: highest rates in Zone B. ⁸⁶	1973-77	Possible reporting bias. Lack of consistency.
Eight children and four pregnant women with skin lesions; 10 adult controls	Chromosome aberrations	No significant increase in peripheral lymphocytes. ⁸³	1976	No suitable controls available. Small size.
145 Zone A residents, 69 plant workers, and 87 controls	Chromosome aberrations	Suggestive, nonsignificant increase in Zone A residents and plant workers. ⁸⁷	1976-77	Large variation mainly accounted for by interobserver differences.
Induced abortions in TCDD-exposed ($n = 19$) and nonexposed ($n = 16$) women	Chromosome aberrations	No increase in exposed maternal blood and placenta. Possible increase in exposed fetal tissues. ⁸⁸	1976-79	Technical factors other than exposure relevant to interpretation.

(continued)

APPENDIX (Continued)

Population	Outcome	Results (references)	Period	Remarks
Induced ($n = 30$) and spontaneous ($n = 4$) abortions in exposed women	Birth defects	No indications of mutagenic, teratogenic, and fetotoxic effects ⁸⁰	1976	Incomplete fetal tissues for examination.
Some 15,000 births to TCDD-exposed and nonexposed women	Birth defects	Frequency in exposed not significantly higher. No association of soil contamination levels with type/frequency of defects. ⁸⁰	1977-82	Small size to detect very rare defects.
48 Zone A children and 48 controls	Immune function	Increased complement activity, lymphocyte response to lectins, and number of peripheral lymphocytes in exposed. ⁸⁴	1976-79	Examination repeated every 5-6 months. Poor compliance of controls. Increased immune reactivity followed by immunodepression?
Adults ($n = 117$) and children from Zones A ($n = 81$), B ($n = 112$), and R ($n = 121$)	Enzyme induction	D-Glucuronic acid excretion increased in exposed at all ages. Highest values in chloracne cases. After 1980 return to normal values. ⁸⁵	1976-81	In 1979 Zone B children showed highest excretion levels. High exposure in parts of Zone R suggested.
Children 6-10 years from Zone A ($n = 69$), B ($n = 83$), and R ($n = 241$)	Liver enzyme Lipid metabolism	Slight abnormalities in GGT and ALT activity in boys with highest exposure. Abnormalities disappeared with time. ⁸⁵	1976-82	Examination repeated every year.
152 chloracne cases and 123 controls	Peripheral neuropathy	Increased frequency of clinical and electrophysiological signs of PNS involvement. ⁸⁶	1982-83	No evidence of clinical peripheral neuropathy according to WHO criteria.
Some 150 chloracne cases and a comparison group from an unexposed area	Skin lesion Neurophysiology Liver enzyme Lipid metabolism	No significant differences between groups. No temporal trends. Absence of abnormalities 10 years after exposure. ⁸⁶	1982-85	In 1984, one subject still had chloracne and five had persisting scars.

36 decontamination workers and 36 unexposed controls	Urine Porphyrin Liver enzyme Lipid metabolism Blood count	No significant differences. No changes over time. No association of tests mean values with length of work. ^{97,98}	1980-84	Environmental measures and personal protection to minimize exposure adopted. Preemployment health examination.
20 Zone A residents and 10 unexposed subjects	Serum TCDD Liver function Lipid metabolism Immune function Enzyme induction	In chloracne cases highest TCDD concentration ever reported in humans. Very high concentration also in Zone A residents without chloracne. TCDD not detected in unexposed. No significant alterations in any of the tests. ⁷⁹	1976-85	Small, highly selected sample. Generalization of results premature.
All persons aged 20-74 years living in contaminated zones and some 180,000 reference subjects	Mortality	Increased cardiovascular mortality early after the accident in the most affected zone. Suggested departure from expectations for several cancers. ⁹⁹	1976-86	Probable "disaster" (stress) rather than TCDD effect. Short latency. Ecological definition of exposure.
All persons aged 0-19 years living in contaminated zones and some 60,000 reference subjects	Mortality	Suggestive, nonsignificant increase of leukemia. ⁹⁷	1976-86	Same limitations as above, plus small size.
All persons aged 20-74 years living in contaminated zones and some 180,000 reference subjects	Cancer incidence	Increased occurrence of hepatobiliary cancer, soft tissue sarcoma, and hematologic neoplasms. Decrease of estrogen-dependent tumors. ¹⁰⁰	1977-88	Consistency with existing experimental and human evidence. Short latency. Partial control of confounding.
All persons aged 0-19 years living in contaminated zones and some 60,000 reference subjects	Cancer incidence	Suggestive, nonsignificant increase of myeloid leukemia and thyroid cancer. ⁹²	1977-86	Same limitations as above, plus small size.

REFERENCES

1. U.S. EPA, Interim Procedures for Estimating Risks Associated with Exposures to Mixtures of Chlorinated Dibenzo-*p*-dioxins and Dibenzofurans (CDDs and CDFs) and 1989 Update, EPA/625/3-89/016, Risk Assessment Forum, Environmental Protection Agency, Washington, DC (1989).
2. WHO, Polychlorinated Dibenzo-*para*-dioxins and Dibenzofurans, *Environmental Health Criteria* 88, International Programme on Chemical Safety, World Health Organization, Geneva (1989).
3. CCTN, Parere della Commissione Consultiva Tossicologica Nazionale sui PCDD e PCDF—Valutazione Tossicologica delle Policlorodibenzodiossine (PCDD) e dei Policlorodibenzofurani (PCDF) in Riferimento alla Loro Presenza nell'Ambiente, *Serie Relazioni* 89(3), pp. 5–55, Istituto Superiore di Sanità, Rome (1989).
4. A. di Domenico, Guidelines for the definition of environmental action alert thresholds for polychlorodibenzodioxins and polychlorodibenzofurans, *Regul. Toxicol. Pharmacol.* 11, 8–23 (1990).
5. V. Silano, Case study: Accidental release of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) at Seveso, Italy, in: *Emergency Response to Chemical Accidents—Interim Document 1* (P. H. Jones and A. Gilad, eds.), pp. 167–203, International Programme on Chemical Safety, Regional Office for Europe, World Health Organization, Copenhagen (1981).
6. G. U. Fortunati, The Seveso accident, *Chemosphere* 14, 729–737 (1985).
7. A. di Domenico, V. Silano, G. Viviano, and G. Zapponi, in: *A Report of NATO/CCMS Working Group on Management of Accidents Involving the Release of Dioxins and Related Compounds* (A. di Domenico and A. E. Radwan, eds.), ISTISAN 88/8, pp. 125–134, Istituto Superiore di Sanità, Rome (1988).
8. G. Reggiani, in: *Agent Orange and Its Associated Dioxin: Assessment of a Controversy* (A. L. Young and G. M. Reggiani, eds.), pp. 227–269, Elsevier, Amsterdam (1988).
9. J. Sambeth, What really happened at Seveso, *Chem. Eng.* 90, 44–47 (1983).
10. R. L. Rawls and D. A. O'Sullivan, Italy seeks answers following toxic release, *Chem. Eng. News* 54, 27–35 (1976).
11. A. W. M. Hay, Tetrachlorodibenzo-*p*-dioxin release at Seveso, *Disasters* 1, 289–308 (1977).
12. J. Peterson, Seveso: The event, *Ambio* 7, 232–233 (1978).
13. A. di Domenico, V. Silano, G. Viviano, and G. Zapponi, Accidental release of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) at Seveso, Italy II. TCDD distribution in the soil surface layer, *Ecotoxicol. Environ. Saf.* 4, 298–320 (1980).
14. S. Cerlesi, A. di Domenico, and S. P. Ratti, 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) persistence in the Seveso (Milan, Italy) soil, *Ecotoxicol. Environ. Saf.* 18, 149–164 (1989).
15. M. H. Milnes, Formation of 2,3,7,8-tetrachlorodibenzodioxin by thermal decomposition of sodium 2,4,5-trichlorophenolate, *Nature* 232, 395–396 (1971).
16. L. Canonica, Seveso, Considerazioni e commenti, *Chim. Ind. (Milan)* 59, 87–89 (1977).
17. G. U. Fortunati, in: *Technological Response to Chemical Pollutions* (P. Bonizzoni and S. Meroni, eds.), pp. 47–60, Ufficio Speciale di Seveso, Regione Lombardia, Milan (1985).
18. A. Bonaccorsi, R. Fanelli, and G. Tognoni, In the wake of Seveso, *Ambio* 7, 234–239 (1978).
19. F. Pocchiarri, A. di Domenico, V. Silano, and G. Zapponi, Accidental release of 2,3,7,8-

- tetrachlorodibenzo-*p*-dioxin (TCDD) at Seveso: Assessment of environmental contamination and of effectiveness of decontamination treatments, in: *The Proceedings of the Sixth International CODATA Conference* (B. Dreyfus, ed.), pp. 31-37, Pergamon Press, Elmsford, NY (1979).
20. F. Pocchiari, A. di Domenico, V. Silano, and G. Zapponi, in: *Accidental Exposure to Dioxins—Human Health Aspects* (F. Coulston and F. Pocchiari, eds.), pp. 5-35, Academic Press, New York (1983).
 21. F. P. Foraboschi and G. U. Fortunati, La decontaminazione di estese superfici e lo smaltimento di grandi volumi di materiali a basso inquinamento di diossina, in: *Technological Response to Chemical Pollutions* (P. Bonizzoni and S. Meroni, eds.), pp. 35-45, Ufficio Speciale di Seveso, Regione Lombardia, Milan (1985).
 22. F. Pocchiari, V. Silano, and G. Zapponi, The chemical risk management process in Italy. A case study: The Seveso accident, *Sci. Total Environ.* **51**, 227-235 (1986).
 23. S. Cerlesi, A. di Domenico, and S. P. Ratti, Recovery yields of early analytical procedures to detect 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in soil samples at Seveso, Italy, *Chemosphere* **18**, 989-1003 (1989).
 24. F. Cattabeni, A. di Domenico, and F. Merli, Analytical procedures to detect 2,3,7,8-TCDD at Seveso after the industrial accident of July 10, 1976, *Ecotoxicol Environ. Saf.* **12**, 35-52 (1986).
 25. F. Pocchiari, F. Cattabeni, G. Della Porta, G. U. Fortunati, V. Silano, and G. Zapponi, Assessment of exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in the Seveso area, *Chemosphere* **15**, 1851-1865 (1986).
 26. G. U. Fortunati and V. La Porta, in: *A Report of NATO/CCMS Working Group on Management of Accidents Involving the Release of Dioxins and Related Compounds* (A. di Domenico and A. E. Radwan, eds.), pp. 49-52, ISTISAN 88/8, Istituto Superiore di Sanità, Rome (1988).
 27. A. di Domenico, V. Silano, G. Viviano, and G. Zapponi, Accidental release of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) at Seveso, Italy. I. Sensitivity and specificity on analytical procedures adopted for TCDD assay, *Ecotoxicol Environ. Saf.* **4**, 283-297 (1980).
 28. G. Belli, S. Cerlesi, and S. P. Ratti, in: *Technological Response to Chemical Pollutions* (P. Bonizzoni and S. Meroni, eds.), pp. 129-133, Ufficio Speciale di Seveso, Regione Lombardia, Milan (1985).
 29. S. P. Ratti, G. Belli, A. Lanza, S. Cerlesi, and G. U. Fortunati, The Seveso dioxin episode: Time evolution properties and conversion factors between different analytical methods, *Chemosphere* **15**, 1549-1556 (1986).
 30. V. La Porta, M. Occa, and M. Matteo, in: *Technological Response to Chemical Pollutions* (P. Bonizzoni and S. Meroni, eds.), pp. 135-153, Ufficio Speciale di Seveso, Regione Lombardia, Milan (1985).
 31. G. Belli, S. Cerlesi, E. Milani, and S. P. Ratti, Statistical interpolation model for the description of ground pollution due to TCDD produced in the 1976 chemical accident at Seveso in the heavily contaminated Zone A, *Toxicol. Environ. Chem.* **22**, 101-130 (1989).
 32. G. Belli, G. Bressi, E. Calligarich, S. Cerlesi, and S. P. Ratti, in: *Chlorinated Dioxins and Related Compounds—Impact on the Environment* (O. Hutzinger, R. W. Frei, E. Merian, and F. Pocchiari, eds.), pp. 155-172, Pergamon Press, Elmsford, NY (1982).
 33. A. Cavallaro, G. Tebaldi, and R. Gualdi, Analysis of transport and ground deposition of the TCDD emitted on 10 July 1976 from the ICMESA factory (Seveso, Italy), *Atmos. Environ.* **16**, 731-740 (1982).
 34. S. P. Ratti, G. Belli, A. Lanza, and S. Cerlesi, in: *Chlorinated Dioxins and Dibenzofurans*

- M. A. Noè, A. Nosedà, S. Garattini, C. Binaghi, V. Marazza, F. Pezza, D. Pozzoli, and G. Cicognetti, 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin levels in cow's milk from the contaminated area of Seveso, Italy, *Bull. Environ. Contam. Toxicol.* **24**, 634-639 (1980).
66. A. di Domenico, V. Silano, G. Viviano, and G. Zapponi, Accidental release of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) at Seveso, Italy. V. Environmental persistence of TCDD in soil, *Ecotoxicol. Environ. Saf.* **4**, 339-345 (1980).
67. A. di Domenico, G. Viviano, and G. Zapponi, in: *Chlorinated Dioxins and Related Compounds—Impact on the Environment* (O. Hutzinger, R. W. Frei, E. Merian, and F. Pocchiari, eds.), pp. 105-114, Pergamon Press, Elmsford, NY (1982).
68. S. P. Ratti, G. Belli, G. Bressi, S. Cerlesi, and C. Zocchetti, An empirical model to describe the TCDD distribution on all the territory around Seveso and the time dependence of its parameters, *Chemosphere* **18**, 921-924 (1989).
69. A. di Domenico, S. Cerlesi, and S. P. Ratti, A two-exponential model to describe the vanishing trend of 2,3,7,8-tetrachlorodibenzodioxin (TCDD) in the soil at Seveso, northern Italy, *Chemosphere* **20**, 1559-1566 (1990).
70. R. Hutter and M. Philippi, in: *Chlorinated Dioxins and Related Compounds—Impact on the Environment* (O. Hutzinger, R. W. Frei, E. Merian, and F. Pocchiari, eds.), pp. 87-93, Pergamon Press, Elmsford, NY (1982).
71. M. Philippi, J. Schmid, H. K. Wipf, and R. Hutter, A microbial metabolite of TCDD, *Experientia* **38**, 659-661 (1982).
72. S. Facchetti, A. Fornari, and M. Montagna, Distribution of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in the tissues of a person exposed to the toxic cloud at Seveso, *Adv. Mass Spectrom.* **8B**, 1405-1414 (1980).
73. D. J. Patterson, Jr., L. Hampton, C. R. Lapeza, Jr., W. T. Belser, V. Green, L. Alexander, and L. L. Needham, High-resolution gas chromatographic/high-resolution mass spectrometric analysis of human serum on a whole-weight and lipid basis for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, *Anal. Chem.* **59**, 2000-2005 (1987).
74. D. G. Patterson, Jr., W. E. Turner, L. R. Alexander, S. Isaacs, and L. L. Needham, The analytical methodology and method performance for the determination of 2,3,7,8-TCDD in serum for the Vietnam veteran Agent Orange validation study, the Ranch Hand validation and half-life studies, and selected NIOSH worker studies, *Chemosphere* **18**, 875-882 (1989).
75. D. G. Patterson, Jr., P. Fürst, L. R. Alexander, S. G. Isaacs, W. E. Turner, and L. L. Needham, Analysis of human serum for PCDDs/PCDFs: A comparison of three extraction procedures, *Chemosphere* **19**, 89-96 (1989).
76. D. G. Patterson, Jr., W. E. Turner, S. G. Isaacs, and L. R. Alexander, A method performance evaluation and lessons learned after analyzing more than 5,000 human adipose tissue, serum, and breast milk samples for polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs), *Chemosphere* **20**, 829-836 (1990).
77. P. Mocarelli, F. Pocchiari, and N. Nelson, Preliminary report: 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin exposure to humans—Seveso, Italy, *Morbid. Mortal. Weekly Rep.* **37**, 733-736 (1988).
78. P. Mocarelli, D. G. Patterson, Jr., A. Marocchi, and L. L. Needham, Pilot study (Phase II) for determining polychlorinated dibenzo-*p*-dioxin (PCDD) and polychlorinated dibenzofuran (PCDF) levels in serum of Seveso, Italy, residents collected at the time of exposure: Future plans, *Chemosphere* **20**, 967-974 (1990).
79. P. Mocarelli, L. L. Needham, A. Marocchi, D. G. Patterson, P. Brambilla, P. M. Gerthoux, L. Meazza, and V. Carreri, Serum concentrations of 2,3,7,8-Tetrachlorodibenzo-*p*-

- dioxin and test results from selected residents of Seveso, Italy. *J. Toxicol. Environ. Health* 32, 357-366 (1991).
80. F. Caramaschi, G. Del Corno, C. Favaretti, S. E. Giambelluca, E. Montesarchio, and G. M. Fara, Chloracne following environmental contamination by TCDD in Seveso, Italy, *Int. J. Epidemiol.* 10, 135-143 (1981).
81. G. Del Corno, C. Favaretti, F. Caramaschi, S. E. Giambelluca, E. Montesarchio, F. Bonetti, and C. Volpato, Distribution of chloracne cases in the Seveso area following contamination by TCDD, *Ig. Mod.* 77, 635-658 (1982).
82. G. M. Reggiani, in: *Halogenated Biphenyls, Terphenyls, Naphthalenes, Dibenzodioxins and Related Products* (R. D. Kimbrough and A. A. Jensen, eds.), pp. 445-470, Elsevier, Amsterdam (1989).
83. S. E. Giambelluca, C. Favaretti, G. Del Corno, F. Caramaschi, E. Montesarchio, F. Bonetti, and C. Volpato, Chloracne and clinical impairment in a group of subjects over 14 years of age, exposed to TCDD in the Seveso area, *Ig. Mod.* 77, 675-680 (1982).
84. G. Filippini, B. Bordo, P. Crenna, N. Massetto, M. Musicco, and R. Boeri, Relationship between clinical and electrophysiological findings and indicators of heavy exposure to 2,3,7,8-tetrachlorodibenzo-dioxin, *Scand. J. Work Environ. Health* 7, 257-262 (1981).
85. G. Ideo, G. Bellati, A. Bellobuono, and L. Bisanti, Urinary D-glucuronic acid excretion in the Seveso area, polluted by tetrachlorodibenzo-p-dioxin (TCDD): Five years of experience, *Environ. Health Perspect.* 60, 151-157 (1985).
86. L. Bisanti, F. Bonetti, F. Caramaschi, G. Del Corno, C. Favaretti, S. Giambelluca, E. Marni, E. Montesarchio, V. Puccinelli, G. Remotti, C. Volpato, and E. Zambrelli, Experience of the accident of Seveso, *Acta Morphol. Acad. Sci. Hung.* 28, 139-157 (1980).
87. L. De Carli, A. Mottura, F. Nuzzo, G. Zei, M. Tenchini, M. Fraccaro, B. Nicoletti, G. Simoni, and P. Mocarelli, Cytogenetic investigation of the Seveso population exposed to TCDD, in: *Plans for clinical and epidemiologic follow-up after area-wide chemical contamination*, Proceedings of an International Workshop, pp. 292-317, National Academy Press, Washington, DC (1982).
88. M. L. Tenchini, C. Grimaudo, G. Pacchetti, A. Mottura, S. Agosti, and L. De Carli, A comparative cytogenetic study on cases of induced abortions in TCDD-exposed and non-exposed women, *Environ. Mutagen.* 5, 73-85 (1983).
89. H. Rehder, L. Sanchioni, F. Cefis, and A. Gropp, Pathologisch-embryologische Untersuchungen an abortusfällen im Zusammenhang mit dem Seveso-Unglück, *Schweiz. Med. Wochenschr.* 108, 1617-1625 (1978).
90. P. P. Mastroiacovo, A. Spagnolo, E. Marni, L. Meazza, R. Bertolini, and G. Segni, Birth defects in the Seveso area after TCDD contamination, *Am. Med. Assoc.* 259, 1668-1672 (1988).
91. P. A. Bertazzi, C. Zocchetti, A. C. Pesatori, S. Guercilena, D. Consonni, A. Tironi, and M. T. Landi, Mortality of a young population after accidental exposure to 2,3,7,8-tetrachlorodibenzodioxin, *Int. J. Epidemiol.* 21, 118-123 (1992).
92. A. C. Pesatori, D. Consonni, A. Tironi, M. T. Landi, C. Zocchetti, and P. A. Bertazzi, Cancer morbidity (1977-1986) of a young population living in the Seveso area, in: *Dioxin '92: Toxicology, Epidemiology, Risk Assessment and Management, Organohalogen Compounds*, Vol. 10, pp. 271-273, Finnish Institute of Occupational Health, Helsinki (1992).
93. S. Barbieri, C. Pirovano, G. Scarlato, P. Turchini, A. Zappa, and M. Maranzana, Long-term effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin on the peripheral nervous system:

- Clinical and neurophysiological controlled study on subjects with chloracne from the Seveso area. *Neuroepidemiology* 7, 29-37 (1988)
94. G. G. Sirchia, in: *Plans for clinical and epidemiologic follow-up after area-wide chemical contamination*, Proceedings of an International Workshop, pp. 234-266, National Academy Press, Washington, DC (1982)
 95. P. Mocarelli, A. Marocchi, P. Brambilla, P. M. Gerthoux, D. S. Young, and N. Mantel. Clinical laboratory manifestations of exposure to dioxin in children. A six-year study of the effects of an environmental disaster near Seveso, Italy. *J. Am. Med. Assoc.* 256, 2687-2695 (1986).
 96. G. Assennato, D. Cervino, E. A. Emmett, G. Longo, and P. Merlo. Follow-up of subjects who developed chloracne following TCDD exposure at Seveso. *Am. J. Ind. Med.* 16, 119-125 (1989)
 97. I. Ghezzi, P. Cannatelli, G. Assennato, F. Merlo, P. Mocarelli, P. Brambilla, and F. Sicurello. Potential 2,3,7,8-tetrachlorobenzo-p-dioxin exposure of Seveso decontamination workers. A controlled prospective study. *Scand. J. Work Environ. Health* 8, (Suppl 1), 176-179 (1982)
 98. G. Assennato, P. Cannatelli, P. Emmett, I. Ghezzi, and P. Merlo. Medical monitoring of dioxin clean-up workers. *Am. Ind. Hyg. Assoc. J.* 11, 586-692 (1989)
 99. P. A. Bertazzi, C. Zocchetti, A. C. Pesatori, S. Guercilena, M. Sanarico, and L. Radice. Ten-year mortality study of the population involved in the Seveso incident in 1976. *Am. J. Epidemiol.* 129, 1187-1200 (1989)
 100. P. A. Bertazzi. Industrial disasters and epidemiology. A review of recent experiences. *Scand. J. Work Environ. Health* 15, 85-100 (1989)
 101. P. A. Bertazzi, A. C. Pesatori, D. Consonni, A. Tironi, M. T. Landi, and C. Zocchetti. Cancer incidence in a population accidentally exposed to 2,3,7,8-tetrachlorodibenzo-para-dioxin. *Epidemiology* 4, 398-406 (1993)
 102. S. Safe, B. Astroff, M. Harris, T. Zacharewski, R. Dickerson, M. Romkes, and L. Biegel. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) and related compounds as antiestrogens: Characterization and mechanism of action. *Pharmacol. Toxicol.* 69, 400-409 (1991).
 103. M. A. Fingerhut, W. E. Halperin, D. A. Marlow, L. A. Piacitelli, P. A. Honechar, M. H. Sweeney, A. L. Glette, P. A. Dill, K. Steenland, and A. Suruda. Cancer mortality in workers exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin. *N. Engl. J. Med.* 324, 212-218 (1991)
 104. L. L. Needham, P. Mocarelli, D. G. Patterson, Jr., A. Marocchi, P. Brambilla, P. M. Gerthoux, L. Meazza, and V. Careri. Findings from our Seveso study and comparisons with other exposed populations. Presented at the 12th International Symposium on Dioxins and Related Compounds "DIOXIN '92," August 24-28, Tampere, Finland (1992)

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